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Assessing the Use of Diurnal Resting Shelters by *Culiseta melanura* (Diptera: Culicidae)

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Abstract

Twenty resting shelters were set on the edge of a known *Culiseta* breeding habitat in four groups of five to support a 4 × 4 Latin square field experiment. Collection times were 0900, 1100, 1300, and 1500 hours and systematically rotated for the order by which each group of five boxes was collected. Mosquitoes were collected from resting shelters by chloroform anesthetization. Collections were identified to species, sex, and physiological status of the females (nonblooded or blood-fed and gravid). More than 77% of the mosquitoes collected were *Culiseta melanura* (Coquillett). Analyses included means and SE for total collections and shelter-day (number collected per units) and means comparison by *t*-test and general linear model with Student–Newman–Keuls or least significant differences means tests for replicate, group, time, and interactions of time and group. There were few significant differences among or between shelter-day means but more blood-fed and gravid female *Cs. melanura* were collected at 1300 hours than any other time. Results confirm the effectiveness of resting shelters in a surveillance program for *Cs. melanura*, demonstrate the ßexibility of resting shelters as a surveillance tool, and suggest that *Cs. melanura* will move to more acceptable resting sites during daylight.

Keywords

Culiseta melanura; resting shelters; time; Latin square

The primary vector of eastern equine encephalitis (EEE) virus in North America is *Culiseta melanura* (Coquillett) (Diptera: Culicidae). Adults are active at night and seek dark, sheltered sites to rest during the day. When placed in dark shaded areas with limited understory vegetation the diurnal resting shelter (RS) is considered an effective collection method to monitor populations of *Culiseta* and other nocturnal mosquitoes (Crans 1995). RSs have been used as a surveillance tool for *Cs. melanura* and EEE virus in New Jersey and central New York state (NYS) for >30 yr (Crans 1995, Howard et al. 2008). In central New York, mosquito and virus surveillance activities are conducted by local health departments with guidance from scientists attached to the NYS Department of Health (NYSDOH). In the four-county area, Madison, Oneida, Onondaga, and Oswego counties rely on seasonal employees to conduct mosquito and virus surveillance.

Oswego Co. seasonal employees and NYSDOH personnel use the Shad Slade Encephalitis Field Station (Morris et al. 1980) as their work station during the mosquito season. The station is on the northern edge of the Toad Harbor-Big Bay Swamp (THS) complex, one of the two enzootic foci of EEE virus in the four-county area (Howard et al. 1996). The THS complex lies along the north shore of Oneida Lake in the towns of Constantia and West

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Monroe in the southeastern corner of Oswego Co. Equine cases of EEE have been reported in 16 of 22 towns in Oswego Co. and routinely occur within an area of 1,000 km^2 (approximately one third) of the total area of the county.

One of the challenges in designing an effective and efficient surveillance system is to establish efficient routes of travel that will cover a large area with employees limited to a 7-h work day and a 35-h work week. Surveillance is conducted with miniature light traps baited with CO_2 and with diurnal RSs. The generally accepted surveillance practice calls for the setting of light traps as late in the day as possible, and retrieving them as early in the morning as possible, to maximize the dry ice and extend battery life. There is no study that has clearly established preferred times for collecting maximum numbers of *Culiseta* or other species from RSs. This study was designed to answer that question.

Materials and Methods

The experiment was designed asa4by4 Latin square field experiment. Twenty RSs were set as four groups of five on the west side of a peninsula that extends 0.3 km eastward into THS from Toad Harbor Road. The area is shown in fig. 1 of Molaei et al. 2006. The west side of the peninsula is dominated by a stand of mature eastern hemlock (*Tsuga canadensis* L.) and American beech (*Fagus grandifolia* Ehrh.). The first two rows were set 40 m east of the long-term RS site (43° 16′01″ N, 76° 05′24″ W) that has been used as an EEE virus surveillance and research study site for >30 yr (Howard et al. 2008). The other two groups were set 30 m from the first two rows. The front rows were 7 m from the wet edge of THS and the second row was 7 m behind the first group. Individual RSs in each row were separated by a minimum of 2 m, and all 20 RSs were set with the box opening facing the swamp (230°). Box groups were named by their position relative to the swamp edge as lower (closest to the wet edge), and upper; and as either left or right relative to an observer (J.J.H.) standing between the two groups and facing the swamp. The four sets of five RSs were designated as lower right (LR), upper right (UR), lower left (LL), and upper left (UL). Chosen collection times were 0900, 1100, 1300, and 1500 hours. The collection times for box groups were randomly assigned on day 1 of each experiment (Table 1). Collection order for the next 3 d was systematically rotated by shifting the site-time assignment from the previous day diagonally down and to the left. The random assignment of group and time was repeated at the start of each 4-d experimental period, but the rows were shifted in the opposite direction from the previous scheme (Table 1).

Mosquitoes were collected from each RS set by chloroform anesthetization. Collections from each group (five RSs) were combined into one 120-ml collection bottle fitted with a plastic snap cap and labeled with the group and time. Bottles were transported to the station where they were placed on dry ice. Specimens were identified to species, by sex, and physiological status of the females: nonblooded or blood-fed and gravid females. Although blood-fed versus gravid specimens of *Culiseta* and *Anopheles* can easily be distinguished, these two physiologic conditions were grouped in our study. Numbers of each were recorded on data sheets that included group, time, date, and number of RSs (units) collected. A data set containing all collection variables was created in Statistical Analysis System (SAS) and analyzed using version 9.2 for personal computers (SAS Institute 2007). Analyses included means and standard errors for total collections and shelter-day (SD, number collected per group/units) means (SDMs). Means comparisons ($P = 0.05$) were by *t*-test and general linear model (GLM) with Student–Newman–Keuls or least significant difference means tests for replicate, group, time, and interactions of time and group.

Results

Two 4-d replicates were completed between 14 and 17 July and 4–7 August 2009 (Table 1). There were 4,274 mosquitoes of five species in three genera that included 3,308 (77.4%) *Cs. melanura*, 696 (16.3%) *Anopheles punctipennis* (Say), and 225 (5.3%) *Anopheles quadrimaculatus* Say s.l. (Table 2). The other two species collected were 28 (1%) *Culiseta morsitans* Dyar and 17 (<1%) *Culex territans* Walker.

Comparison of total collected and SDMs were conducted on the variables nonblooded, blood-fed and gravid females, total females, and males of *Cs. melanura*, total *Cs. melanura*, and total mosquitoes collected. Means (GLM, $n = 8$; *t*-test, $n = 16$) were compared for replicates, defined as weeks 1 and 2, for collection days 1–4, RS group (LR, UR, LL, and UL), groups combined by position relative to the swamp (LR + LL versus UR + UL), by left verses right group, and by time of collection. Of the 48 mean comparisons(means for total collected and SDMs for the six variables listed above), there were only six SDMs that were significant. The SDMs for nonblooded and for total female *Cs. melanura* were higher for the first replicate than the second ($t = 2.28$, $P = 0.03$ and $t = 2.11$, $P = 0.04$, respectively). The SDM for blood-fed and gravid female *Cs. melanura* was higher at the LR site than at the other three sites ($P < 0.05$; GLM), and was higher for the 10 RSs in the right group compared with the left group ($t = 2.63$, $P = 0.01$). There were no other significant differences between and among groups, and these differences did not result in significant differences in SDMs by time of collection (Table 3).

There were observable trends in these data among collection times. Means of all six variables increased between 0900 and 1100 hours (Table 3). Increases ranged from 65% for nonblooded females to 133% for male *Culiseta*. The SDMs for total mosquitoes collected doubled between the two time periods. Collections at 1300 hours were the highest of any time period for all variables, with the exception of non-blooded females and total female *Cs. melanura*, which were 11 and 2% lower, respectively, than the SDMs at 1100 hours. Collections at 1500 hours were less than collections at 1100 or 1300 but higher than collections at 0900 hours. Collections at 0900 were the lowest of the four collection times (Table 3).

For collection day, the SDMs were highest during day 3 (16 July + 6 August) for all variables except blood-fed and gravid *Cs. melanura* than any other day (Table 4). More blood-fed and gravid *Cs. melanura* were collected on day 4 (17 July + 7 August) than any other day. Day 4 SDMs were higher than either day 1 or 2. However, there were no significant differences between SDMs for any of the collection variables when SDMs from days 1 and 2 were compared with days3and4,or days 1 and 3 were compared with days 2and4($P > 0.05$, *t*-test; $n = 16$).

Discussion

We have used resting boxes for surveillance and research of *Cs. melanura* and EEE virus for >30 yr (Howard et al. 2008). However, this is the initial study of the relationship between collection time and number of mosquitoes collected. Although this study supports the observation of Crans (1995) that surveillance is best conducted between 1000 and 1400 hours, data on the numbers of mosquitoes collected per hour were not provided. Crans (1995) stated, “We found mosquito populations build steadily within the boxes during the morning hours and remain static until mid-afternoon.” Although maximum numbers were collected at 1300 hours in our study, sufficient numbers of nonblooded, blood-fed and gravid females, and males of *Cs. melanura* were collected at 0900, 1100, and 1500 hours to support acceptable adult mosquito surveillance data (population numbers, age, and

physiological status, and/or virus assay; Centers for Disease Control and Prevention 1993). Results were expressed as SDMs, which is the equivalent of the more familiar trap-night terminology used with miniature light traps, for comparison with other studies (Morris et al. 1980b, Howard et al. 2008), and with RS data reported by other states in the northeast, particularly those programs, such as New Jersey (Crans 1995), using a number of boxes other than 10 at a site.

A main use of RSs has been for the collection of blood-fed *Cs. melanura* to determine host bloodsource. From the development of large resting shelters (Goodwin 1942) to study *Anopheles*, there have been studies on modifications of resting shelters (such as those by Edman et al. 1968, Morris 1981) and on the use of these shelters to collect blood-fed *Cs. melanura* to determine the host source of blood used by this species and by other mosquito species. The advancements toward specificity of mosquito blood testing procedures, from serologic methods (Nasci and Edman 1981, Morris et al. 1980b) to enzyme-linked immunosorbent assay (Apperson et al. 2004) to molecular-based techniques to obtain species level identifications of hosts of bloodmeals (Molaei et al. 2006), have contributed to but not quite defined the host bloodmeal sources of *Cs. melanura* and the ecology of EEE virus.

On a practical basis our study demonstrates the ßexibility offered by RSs for an EEE virus surveillance program staffed by seasonal employees with hour limitations on their daily work schedules. In addition, over the years, we have found it easier to train employees, especially those with limited or no scientific background, to correctly identify the limited numbers of species (two species each of *Culiseta*, *Anopheles*, and *Culex*) collected in RSs compared with numerous species collected with light traps.

We continue to use the rectangular, all black RS of Morris (1981). The only significant modification we have made to his basic design is the addition of a lid lip made from 5-mm (0.25-in.) fir stripping. Strips are tacked to the inside of the lid and conform to the inside dimensions of the box opening. The strips reduce the likelihood of lid warping and provide a tighter seal for the anesthetization of resting mosquitoes. We have found the construction of RSs from a combination of plywood backs and Masonite sides and lids to be very durable. The current (2010) cost of materials (excluding labor) is ≈US$8.00 per shelter (W.K. Gall, personal communication) and shelters have been used for five to 10 field seasons, translating the cost to US$1–2 per shelter-year. Other researchers have suggested RSs made from less expensive or more readily available products, such as peat or fiber pots (Komar et al. 1995, Montgomery et al. 2006), and Coroplast collapsible plastic boxes (Graham and Turmel 2009). Ten RSs are used at each of our sites, and mosquitoes from each site are collected 2 d/wk. A site considered to be productive for mosquito and arbovirus surveillance purposes (Howard et al. 1996) produces a pool (a minimum of 10 and up to 60) of nonblooded and/or blood-fed and gravid *Cs. melanura* per week. More specimens can be collected by increasing the number of RSs at a site (Crans 1995) or by increasing the frequency of collection to >2 d/wk. In our study there was no difference in the SDMs for RSs collected 2 d/wk versus RSs collected four consecutive days. Ideally, our study design should have been conducted once a month from May to September, the typical adult *Cs. melanura* activity period in central New York (Howard et al. 2008).

The experiments of Morris (1981) showed that the efficient shapes, colors, and orientation for the collection of *Culiseta* were black rectangular RSs with openings that faced west, with the exception that RSs placed in sight of the edge of the swamp be faced into the swamp. Our experiment was conducted with RSs facing into the west and toward the swamp. However, the most critical and limiting factor for a good RS site is a dark area with limited or no understory vegetation. Resting shelters are not traps and mosquitoes can freely move

into and out of the RSs as environmental conditions change. We have referred to RSs as passive surveillance devices (Howard et al. 2008). Our finding that collections increased between 0900 and 1300 indicates that *Cs. melanura* will move to more acceptable resting sites during the day.

We assume that RSs mimic the dark crypts under tree hummocks that are the preferred resting sites for *Cs. melanura.* The swamp or wet woods vegetation within view of these RS groups are dominated by cinnamon ferns (*Osmunda cinnamomea* L.), common highbush blueberries (*Vaccinium corymbosum* L.), and scattered treed hummocks of single or multiple red maples (*Acer rubrum* L.), sometimes paired with yellow birch (*Betula lutea* Michx.) or other hardwood species found in these wet woods (Morris et al. 1980a). As the sun rises, the swamp becomes considerably brighter than the peninsula, which is shaded by the mature hemlocks and American beech. The increases in numbers collected between 0900 and 1300 hours suggests that as the swamp brightens and the temperature increases relative to the cool darkness of the peninsula, mosquitoes resting on swamp vegetation will take ßight to seek more acceptable resting sites. Exactly how they find or “select” a particular RS is unknown. We found no differences in SDMs between the groups of RSs on the edge of the swamp (the lower groups) versus the upper groups, an indication that all four box groups represented acceptable resting sites for *Cs. melanura.*

When sites for RSs can be located, the use of RS for the surveillance of *Cs. melanura* and EEE virus are worthwhile. Of the 580 isolations of EEE virus reported in 2009 to the Centers for Disease Control and Prevention, ArboNet, 73% (n = 422) were from mosquitoes collected in the northeast states (http://diseasemaps.usgs.gov/eee_nc_mosquito.html), lead by Connecticut with 122, New Jersey with 118, New Hampshire with 67, New York with 59, Massachusetts with 54, and Maine with 2. Of all isolations, the earliest in the northeast was from a pool of 28 blood-fed and gravid *Cs. melanura* collected in RSs located on the edge of THS, Oswego County, NY, on 1 July 2009 (NYSDOH, 2009 Arbovirus Surveillance Annual Report, January 2010; J.J.H., unpublished data).

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Table 1

Resting shelter site collection schemes for time of day Latin square experimental design, Toad Harbor Swamp, Oswego Co., NY

Day	Time (h)			
	0900	**1100**	**1300**	**1500**
Replicate 1[a]				
1[b]	LR[c]	LL	UL	UR
2	LL	UL	UR	LR
3	UL	UR	LR	LL
4	UR	LR	LL	UL
Replicate 2[d]	0900	1100	1300	1500
1	UR	LR	UL	LL
2	LL	UR	LR	UL
3	UL	LL	UR	LR
4	LR	UL	LL	UR

[a] Replicate 1 was conducted 14–17 July 2009, earliest sunrise on day 1 = 0538 EDT and earliest sunset on day 4 = 2040 EDT.

[b] Day 1 positions were determined by random selection of site and time for each replicate. Positions for days 2–4 were determined by shifting sites one cell down and to the left for replicate 1; or down and to the right for replicate 2.

[c] Site designations: LR, lower right; LL, lower left; UL, upper left; and UR, upper right.

[d] Replicate 2 was conducted 4–7 August 2009, earliest sunrise on day 1 = 0559 EDT and earliest sunset on day 4 = 2018 EDT.

Table 2

Summary statistics of *Cs. melanura*, *An. quadrimaculatus*, and *An. punctipennis* by physiological status or sex collected from 32 groups of five resting shelters each, during two 4-d periods between 14 and 17 July and 4 and 7 August, 2009

Species, physiological status or sex	No. collected	% total	SDM[a] (mean ± SE)
Cs. melanura, nonblooded ♀	788	23.8[b]	4.93 ± 0.98
Cs. melanura, blood-fed and gravid ♀	264	8.0	1.65 ± 0.20
Cs. melanura, total ♀	1,052	31.8	6.58 ± 1.11
Cs. melanura, ♂	2,256	68.2	14.10 ± 3.34
Cs. melanura, total	3,308	77.4[c]	20.68 ± 4.40
An. quadrimaculatus, nonblooded ♀	76	33.8	0.48 ± 0.08
An. quadrimaculatus, blood-fed and gravid ♀	133	59.1	0.83 ± 0.13
An. quadrimaculatus, ♂	16	7.1	0.10 ± 0.03
An. quadrimaculatus, total	225	5.3[c]	1.41 ± 0.17
An. punctipennis, blood-fed and gravid ♀	207	29.7	1.29 ± 0.17
An. punctipennis, nonblooded ♀	346	49.7	2.16 ± 0.26
An. punctipennis, ♂	143	20.5	0.89 ± 0.21
An. punctipennis, total	696	16.3[c]	4.35 ± 0.56
Total mosquitoes all species	4,274		26.72 ± 4.60

[a] SD, number collected per group/number of shelters.

[b] Number collected/total collected for species.

[c] Number collected/total collected for all species.

Table 3

SDM (n = 8), SE, and percentage of increase or decrease (% Δ) compared with previous collection time for *Cs. melanura* and total mosquitoes collected at 0900, 1100, 1300, and 1500 h

***Cs. melanura* physiological status or sex**	**Collection time (h)**										
	0900		**1100**			**1300**			**1500**		
	SDM	**SE**	**SDM**	**SE**	**% Δ**	**SDM**	**SE**	**% Δ**	**SDM**	**SE**	**% Δ**
Nonblooded ♀	3.78	1.13	6.23	2.56	+65	5.55	2.55	−11	4.15	1.45	−25
Blood-fed and gravid ♀	0.95	0.15	1.80	0.42	+89	2.35	0.43	+30	1.50	0.40	−36
Total ♀	4.73	1.09	8.03	2.92	+70	7.90	2.90	−2	5.65	1.63	−29
♂	7.73	2.47	18.03	7.25	+133	20.35	10.74	+13	10.30	2.87	−50
Total *Cs. melanura*	12.45	3.47	26.05	10.12	+109	28.25	13.60	+8	15.95	4.35	−43
Total mosquitoes	16.28	3.49	32.53	10.55	+100	35.05	13.92	+8	23.00	4.96	−47

Table 4

SDMs (n = 8), SE, and percentage of increase or decrease (% Δ) over previous day's collection for *Cs. melanura* and total mosquitoes collected on four consecutive days during two replicates (day 1, 14 July + 4 August; day 2, 15 July + 5 August; day 3, 16 July + 6 August; and day 4, 17 July + 7 August 2009)

***Cs. melanura*, physiological status or sex**	**Collection day**										
	1		**2**			**3**			**4**		
	SDM	**± SE**	**SDM**	**± SE**	**% Δ**	**SDM**	**± SE**	**% Δ**	**SDM**	**± SE**	**% Δ**
Nonblooded ♀	2.95	0.49	3.73	0.84	+26	8.23	2.62	+121	4.80	2.62	−42
Blood-fed and gravid ♀	1.43	0.23	1.23	0.31	−14	1.95	0.47	+59	2.00	0.51	+3
Total ♀	4.38	0.68	4.95	0.96	+13	10.18	2.90	+106	6.80	3.04	−33
♂	8.75	2.57	6.18	1.02	−29	21.13	6.93	+242	20.35	10.79	−5
Total *Cs. melanura*	13.13	3.18	11.13	1.84	−15	31.30	9.79	+181	27.15	13.81	−13
Total mosquitoes	19.23	4.59	16.90	1.87	−12	38.10	10.01	+125	32.63	14.29	−14

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